Intracellular signalling: **Sphingosine-1-phosphate branches out**

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Recent studies indicate that sphingosine-1-phosphate – known to be an important signalling molecule in animal cells – is involved in Ca^{2+}-dependent signalling in yeast and higher plants, raising the likelihood that it is a universal signalling molecule with a diverse range of functions in eukaryotes.

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Intracellular signalling in eukaryotes involves complex interactions between proteins, membrane lipids, soluble messengers and downstream components. Sphingolipids are a major component of membrane lipids, and their derivative sphingosine-1-phosphate has been shown in animal cells to be involved in the regulation of processes as diverse as cell proliferation, chemotaxis, differentiation, stress responses, senescence and apoptosis [1]. But the mode of action of sphingosine-1-phosphate has been poorly understood, both where in the cell it acts and the range of molecules with which it interacts. Sphingosine-1-phosphate is formed from sphingosine by the action of sphingosine kinase, and a range of stimuli are known to increase its levels in animal cells [1]. Sphingosine-1-phosphate levels can be further regulated by the action of sphingosine-1-phosphate phosphatase, which antagonises sphingosine kinase, and sphingosine-1-phosphate lyase, which cleaves sphingosine-1-phosphate into hexadecanal and phosphoethanolamine [1]. Despite our poor understanding of how sphingosine-1-phosphate functions, the case that it is a universally important signalling molecule is strengthened by recent evidence implicating it in Ca^{2+}-dependent signalling in yeast and higher plants [2–4].

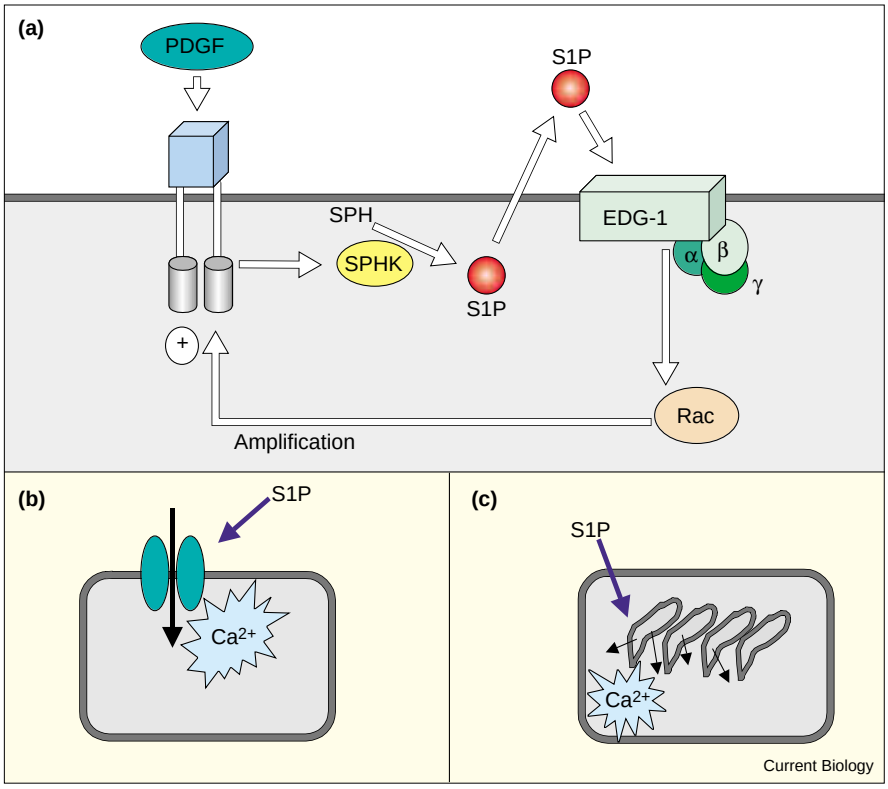
In animals, sphingosine-1-phosphate is the extracellular ligand for G-protein-coupled receptors of the 'endothelial differentiation gene' (EDG) family [1,5]. In human embryonic kidney (HEK) cells, these receptors are involved in cell migration, angiogenesis and vascular maturation [1,5,6]. The *edg-1* gene is essential for migration of vascular mural cells to arteries and capillaries [7]. Interaction of sphingosine-1-phosphate with the EDG-1 receptor leads to activation of a trimeric G protein that regulates lamellipodial protrusion and chemotaxis *via* the small GTP-binding protein Rac (Figure 1). Interestingly, Hobson *et al.* [5] recently showed that sphingosine-1-phosphate is produced in the same cells by stimulation of sphingosine kinase in response to the stimulation of its tyrosine kinase receptor by platelet derived growth factor (PDGF). Rac is also known to act to activate protein kinases involved in chemoreceptor signalling [8] and thus may provide spatially localised amplification of cross talk between tyrosine kinase coupled receptors and G-coupled receptors leading to localised cytoskeletal re-organization during lamellipodium formation during PDGF-directed chemotaxis.

Sphingosine-1-phosphate was also shown in the same study [5] to activate EDG-1 receptors in adjacent cells. A family of cytosolic proteins known as β-arrestins bind to activated G proteins to terminate receptor–G protein coupling, and their translocation to the plasma membrane is known to be stimulated by sphingosine-1-phosphate. In order to investigate whether sphingosine-1-phosphate can mediate communication between cells, the authors monitored the translocation of green fluorescent protein (GFP)-labelled β-arrestin to the plasma membrane in EDG-1-expressing cells that were co-cultured with cells expressing sphingosine kinase and β-arrestin labelled with a red fluorescent protein. They found that sphingosine-1-phosphate production by the red-fluorescing cells induced β-arrestin translocation to the plasma membrane in the green-fluorescing cells, indicating that it might indeed act as a signal in cell–cell communication.

There is also evidence that sphingosine-1-phosphate may directly modulate the production of intracellular signals that are encoded in fluctuations in cytosolic Ca^{2+} levels, though it has been argued [1] that it is difficult to disentangle direct intracellular effects of sphingosine-1-phosphate from those that occur via the activation of a membrane receptor, particularly where exogenous sphingosine-1-phosphate is applied in order to elicit a response. In animal cells, inhibition of sphingosine kinase is known to inhibit Ca^{2+} mobilization from intracellular stores, and sphingosine-1-phosphate can directly stimulate Ca^{2+} release from intracellular stores in permeabilized cells and from isolated microsomal membrane fractions [9]. This appears to operate *via* a novel pathway that does not involve other mediators of Ca^{2+} release such as inositol (1,4,5) trisphosphate (IP_3) or cyclic ADP ribose (cADPR) [9].

Signalling functions for sphingosine-1-phosphate clearly extend beyond those animal responses studied so far. As with animal cells, sphingolipids comprise a major fraction (around 30%) of yeast plasma membrane phospholipids [10]. Though sphingosine-1-phosphate's functions in yeast are largely unexplored, it is implicated in responses to heat stress, and in the regulation of cell proliferation and

Figure 1



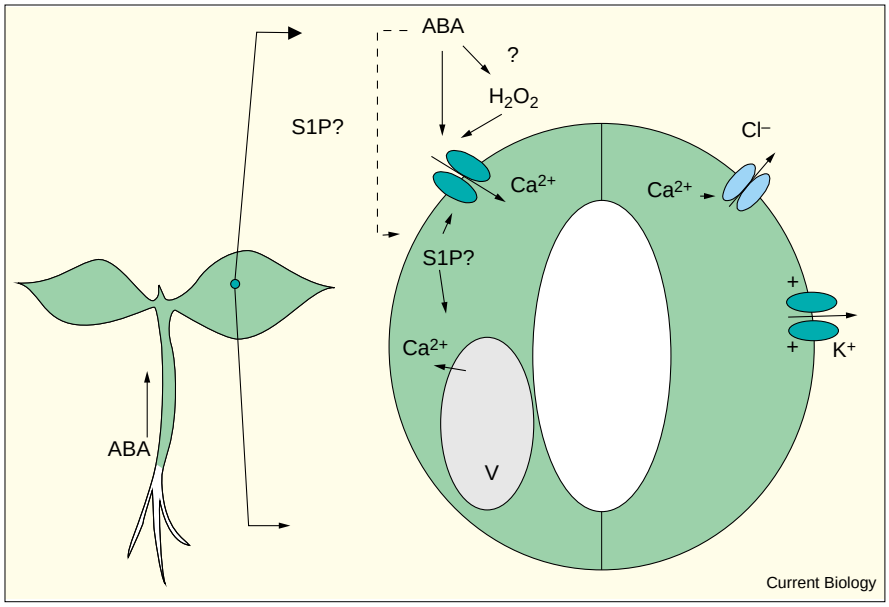
Cellular sites of action of sphingosine-1-phosphate in animals and yeast. (a) Sphingosine-1-phosphate (S1P) is produced from sphingosine (SPH) in response to chemotactic signals (PDGF) acting via a receptor tyrosine kinase, which activates sphingosine kinase (SPHK). Sphingosine-1-phosphate appears in turn to be a ligand for the G-coupled receptor EDG-1, which regulates vascular cell motility. Positive feedback between the G-coupled receptor and the PDGF receptor may lead to localised amplification of chemotactic signals and cytoskeletal reorganization. Less defined roles for SPP include: (b) the activation of calcium channel in the yeast plasma membrane; and (c) stimulation of intracellular calcium release in animal cells.

metabolism [2,3]. Recent work [3] on mutants of the budding yeast *Saccharomyces cerevisiae* that are defective in sphingosine kinase, sphingosine-1-phosphate phosphatase or sphingosine-1-phosphate lyase has provided evidence that sphingosine-1-phosphate plays a part in Ca^{2+} signalling *via* a plasma membrane Ca^{2+} influx pathway. When exogenous sphingosine was added to *S. cerevisiae* cells, increased Ca^{2+} influx was observed, together with elevation of cytosolic Ca^{2+}, monitored with the Ca^{2+}-sensitive photoprotein aequorin. These effects were dependent on the activity of sphingosine kinase. Mutants defective in sphingosine-1-phosphate lyase showed five-fold higher Ca^{2+} accumulation, which was again absent in sphingosine kinase mutants.

Interestingly, the effects of sphingosine addition were stereospecific at low applied concentrations, whereas higher concentrations gave rise to non-specific Ca^{2+} accumulation that was not dependent on sphingosine kinase activity. The pore-forming subunit of the Ca^{2+} influx channel Cch1p was required for the major component of the sphingosine-1-phosphate-induced Ca^{2+} accumulation. This work also showed that Ca^{2+} accumulation in response to sphingosine-1-phosphate production activated a downstream signalling pathway involving the Ca^{2+}-dependent phosphatase calcineurin [3]. There was no evidence in this system that sphingosine-1-phosphate induces the release of Ca^{2+} from intracellular stores. The function of this sphingosine-1-phosphate-stimulated signalling pathway in yeast is not clear. Surprisingly, mutants defective in sphingosine kinase activity were not compromised in their ability to generate Ca^{2+} signals in response to a variety of stimuli, such as mating, high salt, heat shock or hypo-osmotic shock. This suggests that in yeast, at least sphingosine-1-phosphate may be involved in novel signalling processes.

Another notable recent finding on sphingosine-1-phosphate has come from work with higher plants [4]. The stomatal guard cell provides an excellent model for plant signalling studies. Pairs of guard cells in the leaf epidermis form pores that allow the exchange of water and gasses between the plant and the atmosphere. The size of the stomatal pore aperture regulates the amount of water lost from the plant and is determined by the shape of the guard cells, which in turn is determined by changes in turgor arising from fluxes of ions and water in and out of the cells (Figure 2). A variety of external stimuli and plant hormones, such as auxin and abscisic acid (ABA), can modulate these fluxes. ABA produced in the roots during desiccation stress [11] can be transported to the leaves, where it reduces stomatal aperture by allowing efflux of K^+ and Cl^- through plasma membrane channels (Figure 2).

Figure 2



Putative interactions between sphingosine-1-phosphate and ABA during Ca^{2+}-based signalling in guard cells. In this simplified scheme, ABA can activate plasma membrane Ca^{2+} channels; this may involve the production of H_2O_2. Ca^{2+} elevation may be further amplified by release of Ca^{2+} from intracellular stores, notably the vacuole (V). Sphingosine-1-phosphate may modulate these interactions directly or indirectly. The precise sites of action of sphingosine-1-phosphate in this complex signalling pathway remain to be determined. Elevated Ca^{2+} can lead to activation of plasma membrane Cl^- channels, depolarisation of the plasma membrane and efflux of K^+ ions.

The signal transduction pathways that bring about these changes are becoming increasingly clear [12], and involve oscillations of cytosolic Ca^{2+} that may arise in part from the direct activation by ABA of plasma membrane Ca^{2+} channels [13]. A range of other intracellular second messengers, and the production of hydrogen peroxide, are also implicated in this response (Figure 2) [11]. Sphingosine-1-phosphate now joins a list of other mobile messengers known to modulate the ion fluxes that control stomatal movements (Figure 2).

In their recent work, Ng *et al.* [4] showed that sphingosine-1-phosphate could be extracted from leaves of *Commelina communis*. The levels of sphingosine-1-phosphate increased when plants were subjected to drought conditions. The addition of sphingosine-1-phosphate to open stomata in epidermal strip preparations resulted in partial closure; this was not produced by the inactive analogue dihydro-sphingosine phosphate. Using fluorescent dyes to monitor cytosolic Ca^{2+} in single cells, low concentrations of sphingosine-1-phosphate were found to induce small Ca^{2+} oscillations. At higher concentrations sphingosine-1-phosphate induced larger oscillations, but with a different pattern, and also accelerated the rate of stomatal closure. The competitive inhibitor of sphingosine kinase, DL-threo-dihydrosphingosine significantly inhibited the closure of stomata induced by addition of ABA and abolished the sphingosine-1-phosphate-induced Ca^{2+} oscillations.

These results together indicate that sphingosine-1-phosphate may play a role in regulation of guard cell aperture. In this context it is interesting to note that in animal cells sphingosine-1-phosphate activates Ca^{2+}-dependent K^+ channels through the action of a non-receptor tyrosine kinase [14]. A major challenge will be to identify the precise points at which sphingosine-1-phosphate operates in the complex guard cell signalling machinery, and to determine whether these are intracellular or extracellular. If they are extracellular, does sphingosine-1-phosphate operate over short or long distances in plants? This work [4] adds sphingosine-1-phosphate to the growing list of signal transduction components that have been discovered in higher plants from work on guard cells. Further improvements in our knowledge of the role of sphingosine-1-phosphate in higher plants should come from cloning the gene for plant sphingosine kinase, as well as those encoding other proteins involved in regulation of sphingosine-1-phosphate levels. Clearly, as there is no evidence for the presence of EDG-type G-coupled receptors in plants, this work is likely to yield new receptors and interacting molecules in sphingosine-1-phosphate signal transduction pathways.

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